Technical Data Sheet

FITC Rat Anti-Human IL-4

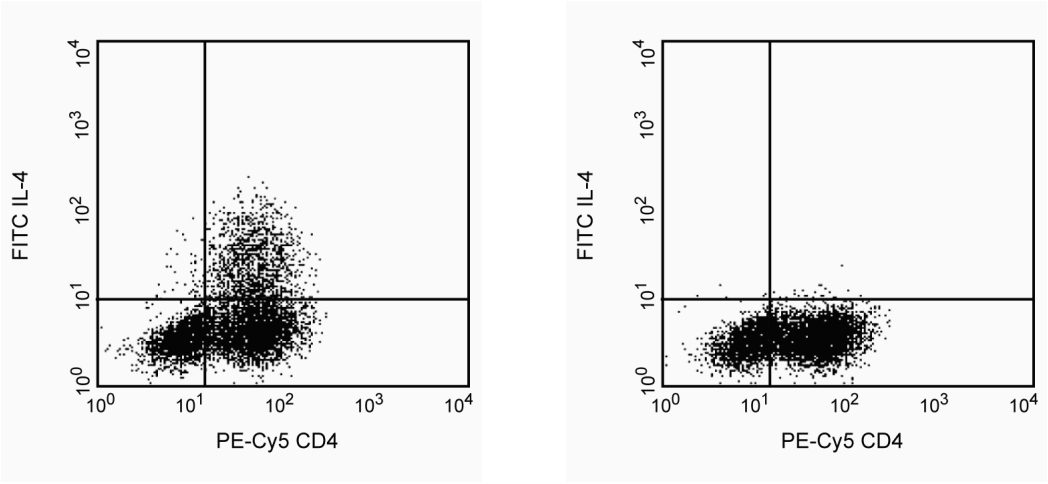
Product Information

Material Number:	**554484**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	MP4-25D2
Immunogen:	Purified Recombinant Human IL-4
Isotype:	Rat IgG1
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The MP4-25D2 antibody reacts with human interleukin-4 (IL-4). The immunogen used to generate the MP4-25D2 hybridoma was purified recombinant human IL-4. This is a neutralizing antibody. The MP4-25D2 antibody has been reported to cross react with IL-4 from rhesus monkeys. The use of the MP4-25D2 antibody for epitope mapping of human IL-4 has been described.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Expression of IL-4 by stimulated human peripheral blood mononuclear cells (PBMC). *Human PBMC were stimulated with soluble anti-human CD3 antibody (1 µg/ml final concentration; Cat. No. 555329), recombinant human IL-2 (10 ng/ml final concentration; Cat. No. 554603) and recombinant human IL-4 (10 ng/ml final concentration; Cat. No. 554605) for 2 days. The cells were subsequently cultured in medium containing recombinant human IL-2 and recombinant human IL-4 for 3 days. Finally, the cells were harvested and stimulated for 6 hours with PMA (Sigma) and calcium ionophore A23187 (Sigma) in the presence of GolgiStop™ (2µM final concentration; Cat. No. 554724). The cells were harvested, stained with PE-Cy5™-anti CD4 (.25 µg final concentration; Cat. No. 555348), fixed, permeabilized, and subsequently stained with 0.12 µg of FITC-rat anti-human IL-4 antibody (FITC-MP4-25D2, Cat. No. 554484) by using the BD Pharmingen staining protocol (left panel). To demonstrate specificity of staining, the binding of FITC-MP4-25D2 antibody was blocked by preincubation of the fixed/permeabilized cells with unlabelled MP4-25D2 antibody (2.5 µg, Cat. No. 554482; right panel) prior to staining. The quadrant markers for the bivariate dot plot were set based on the autofluorescence controls and verified using unlabelled antibody blocking controls.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.
Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

BD Biosciences
bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit bdbiosciences.com/how_to_order/

BD
BD Biosciences

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometry Analysis: The FITC-conjugated MP4-25D2 antibody can be used for multicolor immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-4-producing cells within mixed cell populations (see image, left panel). For optimal immunofluorescent staining for flow cytometric analysis, this antibody should be titrated (≤ 0.5 µg mAb/million cells). A useful control for demonstrating specificity of staining is the following: pre-block the fixed/permeabilized cells with unlabelled MP4-25D2 antibody (Cat. No. 554482) prior to staining. The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable rat IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is FITC-R3-34 immunoglobulin (Cat. No. 554684); use at comparable concentrations to antibody of interest (e.g. , ≤ 0.5 µg mAb/ 1 million cells). For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on intracellular staining in the Immune Function Handbook.

ELISA Detection: The biotinylated MP4-25D2 (Cat. No. 554483) antibody is useful as a detection antibody for a sandwich ELISA that measures human IL-4 protein levels. Biotinylated MP4-25D2 antibody can be paired with the purified 8D4-8 antibody (Cat. No. 554515) as the capture antibody, with recombinant human IL-4 protein (Cat. No. 554605) as the standard. For assay of IL-4 in serum or plasma, the BD OptEIA™ Human IL-4 ELISA Set (Cat. No. 555194) or BD OptEIA™ Human IL-4 ELISA Kit (Cat. No. 550614) is recommended.

Neutralization: The NA/LE format of the MP4-25D2 antibody (Cat. No. 554481) has been reported to be useful for neutralization of human IL-4 bioactivity. A suitable NA/LE™ rat IgG1 isotype control to match the NA/LE™ MP4-25D2 antibody is the R3-34 antibody, Cat. No. 554683.

Suggested Companion Products

Catalog Number	Name	Size	Clone
555062	Hick-2 Cytokine Positive Control Cells	5x10^6 cells	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554605	Recombinant Human IL-4	5 µg	(none)
554603	Recombinant Human IL-2	5 µg	(none)
555348	PE-Cy5 Mouse Anti-Human CD4	100 tests	RPA-T4
555329	Purified NA/LE Mouse Anti-Human CD3	0.5 mg	UCHT1
554684	FITC Rat IgG1, κ Isotype Control	0.1 mg	R3-34

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev.* 1992; 127:5-24.(Clone-specific: ELISA, Neutralization)

Chretien I, Van Kimmenade A, Pearce MK, Banchereau J, Abrams JS. Development of polyclonal and monoclonal antibodies for immunoassay and neutralization of human interleukin-4. *J Immunol Methods.* 1989; 117(1):67-71.(Clone-specific: ELISA, Neutralization)

Jung T, Schauer U, Rieger C, et al. Interleukin-4 and interleukin-5 are rarely co-expressed by human T cells. *Eur J Immunol.* 1995; 25(8):2413-2416. (Clone-specific: Flow cytometry)

Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods.* 1995; 188(1):117-128.(Methodology: IC/FCM Block)

Ramanathan L, Ingram R, Sullivan L, et al. Immunochemical mapping of domains in human interleukin 4 recognized by neutralizing monoclonal antibodies. *Biochemistry.* 1993; 32(14):3549-3556.(Clone-specific: Neutralization)